These data suggest that sequences within Area III mediate early pancreas-wide Pdx1 expression. We found that Area III contains a PTF1 binding site, and the pancreas-specific component of this complex, Ptf1a, bound to Area III in vitro. Importantly, Ptf1a occupied Area III of the endogenous Pdx1 promoter in E11.5 pancreatic buds. These data are the first to demonstrate that Pdx1 is a direct target of PTF1 and suggest that PTF1 mediates Pdx1 expression during early pancreas development.

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Program/Abstract # 166
Genome-wide analysis of Nkx2.2 binding sites using ChIP-tag sequencing (ChIP-TS)

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An understanding of pancreas development is emerging, highlighting the roles played by transcription factors such as Pdx-1, Ngn3, Nkx2.2, etc. There is, however, a paucity of information on the physiological targets of these factors. Chromatin immunoprecipation (ChIP) is a technique for identification of transcription factor binding sites using PCR (ChIP-PCR), hybridization to arrays (ChIP-Chip), or sequencing of tags (ChIP-TS). This latter strategy has successfully identified binding sites for p53, Oct4 and Nanog in mouse ES cells. Although ChIP-TS is unbiased and not restricted by our knowledge of transcriptional units, a major disadvantage is the cost associated with obtaining reliable binding site data. We describe here the utilization of the Solexa sequencing device to identify binding sites for the transcription factor Nkx2.2. This approach enables extremely deep sequencing (i.e. ≥ 1 million tags) for a fraction of the cost associated with conventional sequencing technologies. By combining the ChIP-TS data with previously generated serial analysis of gene expression data (www.mouseatlas.org) and our regulatory motif identification pipeline (www.cisred.org) we anticipate that it will be possible to map the DNA binding sites for key transcriptional regulators of pancreas development further unraveling the transcriptional networks regulating pancreas development. This project is funded by Genome Canada, Genome BC, and the BC Cancer Foundation.

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Program/Abstract # 167
Expression and TTF-1-mediated transcriptional control of α5 nAChRs in the developing lung

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Nicotinic acetylcholine receptors (nAChRs) are ligand-gated channels formed by five similar subunits that function in signal transduction, proliferation, and apoptosis. nAChR assembly often includes α_5, a structural subunit detected in many non-neuronal tissues. Prevalence of α_5 subunits and their contribution to signaling during pulmonary development, however, are unknown. α_5 subunits were assessed by immunohistochemistry in mouse lungs from embryonic day (E)13.5 to post-natal day (PN)10. Transcriptional control of α_5 was determined by transfection of murine pulmonary epithelial cell lines with a reporter containing 1.7-kb of the mouse α_5 promoter and TTF-1, a key transcription factor that controls normal branching morphogenesis in the lung. α_5 was initially detected in the most proximal primitive tubules at E15.5. α_5 expression followed the proximal-distal axis and was detected throughout the lung until PN5, a late stage of alveologenesis. From PN5 to PN10, α_5 expression decreased in the proximal airways and was exclusively observed in the peripheral lung by PN10. Co-localization staining revealed that α_5 was expressed in Clara cells in the proximal lung, type II alveolar epithelial cells, and pulmonary vasculature. Promoter mutagenesis revealed that TTF-1 induced the transcription of α_5 via interaction with specific TTF-1 response elements. Exogenous TTF-1 also induced a significant increase in α_5 transcription. These data show that α_5 is specifically controlled in a temporal and spatial manner. Such specific regulation of α_5 in differentiating pulmonary epithelial cells likely indicates involvement in signaling that ensures normal morphogenesis.

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Program/Abstract # 168
Investigating microRNA function in mammalian lung development

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miRNAs are small RNA molecules encoded by the genome, and they function as repressors of gene expression. In mammals, miRNAs are predicted to regulate the expression of ~30% of genes in the genome. They have been shown to play central roles in many developmental processes. To address whether miRNAs function in lung development, we conditionally inactivated DICER, an enzyme that is essential in processing miRNAs to their mature, active forms. Lung-specific inactivation of Dicer leads to an arrest of lung branching morphogenesis, a major event during lung development. We will present data toward understanding the cellular and molecular mechanism of miRNA function in lung formation.

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